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Suppression of the black pigment in female hybrids of *Papilio glaucus* and *P. multicaudatus*: further evidence of the value of ecdysone in breaking pupal diapause.

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Abstract. Using injections of ecdysone it was possible to break indefinite pupal diapause in female hybrids between a black *Papilio g. glaucus* female and a male *P. multicaudatus*. The insects demonstrate well-marked suppression of the black *glaucus* pigment.

In previous papers (Clarke, Sheppard and Willig, 1972; Clarke and Willig, 1977 and West and Clarke, 1988) we showed that the black pigment of *Papilio glaucus* females could be suppressed in varying degrees in hybrids using males of *Papilio rutulus*, *Papilio glaucus canadensis* and *Papilio eurymedon*, and probably also with *Papilio multicaudatus*. However, there was some doubt about the last (see West and Clarke, 1988, page 197) as the two yellow females recorded in a back-cross brood (female black *glaucus* × male F1 *glaucus* × *multicaudatus*) were lost from our collection. The present paper remedies this carelessness.

Materials and Methods

On 11. VII. 1987, using the hand-pairing technique (Clarke, 1952) we crossed a black female *P. glaucus* (ex 1986 stock obtained from Pasadena, Maryland, U.S.A.) with a *P. multicaudatus* male (obtained from pupae sent by David V. McCorkle and originating from Klickitat Co. Washington). The caterpillars (brood 19052) were fed on *Liriodendron*, and produced 13 male butterflies by 1. IX. 1987. A further two male pupae overwintered but only produced deformed insects in April, 1988, i.e. 15 males in all.

We scored 13 of the pupae as being female but none of them eclosed.

Seven were therefore given cold/hot shock treatment (see footnote) some by Mr. Karl Bailey and some by Mr. George Beccaloni, but this was unsuccessful and by 30. III. 1989 we only had six (untreated) pupae left alive. We therefore decided to try the use of ecdysone preparations and one of us (HHR) carried out the injections. The six pupae were divided into two groups of three, one batch being injected with ecdysone and the other with 20-hydroxyecdysone, but the latter was unsuccessful, all the pupae dying, possibly because the action of the hormone was too rapid. To the other three pupae six ecdysone injections, each of 170 ng, were given (as in Clarke and Willig, 1977) on 3, 6, 8, 10, 12 and 14, IV. 1989.

Results

Three females emerged between 29. IV. 1989 and 2. V. 1989 and are shown in Figure 1. It will be seen that only one (No. 1) grew fully, but this is clearly primarily a yellow insect though with some "sooty" features. (Black females normally always produce black daughters and yellow females yellow ones.) The second hybrid (No. 2) failed to grow, but it seems likely that had it done so it would have resembled No. 1. No. 3 is more problematical — it too did not grow and is a good deal blacker, but there is some yellow pigment in the hindwing. Suppression of black therefore is probably variable.

We can therefore safely conclude that the male *multicaudatus* does carry a suppressor of black, probably autosomally controlled (see West and Clarke, 1988) though the expression is variable.

Discussion

It is easy to surmise that the presence of a suppressor would protect the insect in areas where the model *Battus philenor* does not fly and a black mutant would then be disadvantageous. However from an evolutionary point of view these "anticipatory" theories are troublesome, the rather lame explanation being that the "waiting" gene must have been doing "something else". The same problem occurs in Man with certain drugs, for example isoniazid — where there is a precise dimorphism for the rates at which the drug is metabolised. However the gene controlling this must have been present millions of years before isoniazid was synthesised.

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Literature Cited

CLARKE, C. A., SHEPPARD, P. M. & WILLIG, A., 1972. The use of ecdysone to break a

Fig. 1. Fl hybrid females of the cross ♀ black *P. glaucus* X ♂ *P. multicaudatus* (see text).

two and a half year pupal diapause in *Papilio glaucus* female × *Papilio rutulus* male hybrids. The Entomologist, *105*: 137–8

CLARKE, C. A. & WILLIG, A., 1977. The use of α-ecdysone to break permanent diapause of female hybrids between *Papilio glaucus* L. female and *Papilio rutulus* Lucas male. Journal of Research on the Lepidoptera *16*: 245–248

WEST, D.A., & CLARKE, SIR CYRIL A., 1988. Suppression of the black phenotype in females of the *P. glaucus* group (Papilionidae). Journal of Research on the Lepidoptera. *26*: 187–200

CLARKE, C. A., 1952. Hand pairing of *Papilio machaon* in February. Entomologist's Record. *64*: 98–100

Footnote

Details of Mr. Karl Bailey's cold/hot shock therapy.

On 17.XI. 1987 the hybrid pupae were refrigerated at −4.5°C ± .5°C and left for 8 weeks. They were then transferred to an incubator at 26°C in the hope that the diapause would be broken. This did not occur and the pupae died.

Details of Mr. George Beccaloni's cold/hot shock therapy.

Pupae received on 8. II. 1989 and placed in a refrigerator at 0°C where they were kept for three months. They were removed on 7. V. 1989 and subsequently kept at room temperature. They did not emerge and were given our standard ecdysone treatment beginning on 9. VI. 1989. They were then kept at room temperature but all died and no insects had formed up inside them.